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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR § 1.53(b))

Attorney Docket No.	*0942.2570003/RWE/DRM*
First Inventor or Application Identifier	*Hartley*
Title	*Nucleic Acid Markers Ladder for Estimating Mass*
Express Mail Label No.	

09/20/00 JC564 U.S. PTO

09/20/00 JC564 U.S. PTO

APPLICATION ELEMENTS
See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents
Box Patent Application
Washington, DC 20231

☒ * Fee Transmittal Form (*e.g.*, PTO/SB/17)
(Submit an original, and a duplicate for fee processing)

☒ Specification [Total Pages 17]
(preferred arrangement set forth below)
- Descriptive title of the Invention
- Cross References to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings *(if filed)*
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

3. ☒ Drawing(s) (35 U.S.C. 113) [Total Sheets 2]

4. ☒ Oath or Declaration [Total Pages 2]

a. ☐ Newly executed (original or copy)

b. ☒ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional with Box 17 completed)*
[Note Box 5 below]

i. ☐ DELETION OF INVENTOR(S)
Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR §§ 1.63(d)(2) and 1.33(b).

5. ☒ Incorporation By Reference *(useable if Box 4b is checked)*
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Microfiche Computer Program *(Appendix)*

7. Nucleotide and/or Amino Acid Sequence Submission (*if applicable, all necessary)*

a. ☐ Computer Readable Copy

b. ☒ Paper Copy (identical to computer copy)

c. ☒ Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. ☐ Assignment Papers (cover sheet & document(s))

9. ☐ 37 CFR 3.73(b) Statement (when there is an assignee) ☐ Power of Attorney

10. ☐ English Translation Document *(if applicable)*

11. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations

12. ☐ Preliminary Amendment

13. ☒ Return Receipt Postcard (MPEP 503)
(Should be specifically itemized)

14. ☐ *Small Entity Statement(s) *(PTO/SB/09-12)* ☐ Statement filed in prior application, Status still proper and desired

15. ☐ Certified Copy of Priority Document(s)
(if foreign priority is claimed)

16. ☒ Other: 37 C.F.R. § 1.136(a)(3) Authorization (in duplicate)

☒ Other: Request to Open New Disk File

☒ Other: Letter to PTO Draftsman: Submission of Formal Drawings (in duplicate) with 2 sheets of Formal Drawings (Figs. 1 and 2)

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17. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below and in a preliminary amendment:*

☒ Continuation ☐ Divisional ☐ Continuation-in-Part (CIP) of prior application No: 09/114,911

Prior application information: *Examiner: Houtteman, S.* *Group/Art Unit: 1656*

18. CORRESPONDENCE ADDRESS

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SIGNATURE	Robert W. Esmond	*Date*	September 20, 2000

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FEE TRANSMITTAL
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Patent fees are subject to annual revision.
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See 37 C.F.R. §§ 1.27 and 1.28.

TOTAL AMOUNT OF PAYMENT	($) 690.00

Complete if Known	
Application Number	To be assigned
Filing Date	HEREWITH
First Named Inventor	Hartley
Examiner Name	To be assigned
Group / Art Unit	To be assigned
Attorney Docket Number	0942.2570003/RWE/DRM

METHOD OF PAYMENT (check one)

1. ☐ The Commissioner is hereby authorized to charge indicated fees and credit any overpayment to:

Deposit Account Number	19-0036
Deposit Account Name	Sterne, Kessler, Goldstein & Fox P.L.L.C.

☐ Charge Any Additional Fee Required Under 37 CFR §§ 1.16 and 1.17

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*Charge any deficiencies or credit any overpayments in the fees or fee calculations of Parts 1, 2 and 3 below to Deposit Account No. 19-0036.

FEE CALCULATION

1. BASIC FILING FEE

Large Fee Code	Entity Fee ($)	Small Fee Code	Entity Fee ($)	Fee Description	Fee Paid
101	690	201	345	Utility filing fee	690.00
106	310	206	155	Design filing fee	
107	480	207	240	Plant filing fee	
108	690	208	334	Reissue filing fee	
114	150	214	75	Provisional filing fee	

SUBTOTAL (1) ($) 690.00

2. EXTRA CLAIM FEES

	Extra	Fee from below		Fee Paid
Total Claims 14 - 20** =	0	X	=	00.00
Indep. Claims 3 - 3** =	0	X	=	00.00
Multiple Dependent			=	00.00

** or number previously paid, if greater; For Reissues, see below

Large Fee Code	Entity Fee ($)	Small Fee Code	Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	78	202	39	Independent claims in excess of 3
104	260	204	130	Multiple dependent claim
108	78	209	39	**Reissue independent claims over original patent
110	18	210	9	**Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Fee Code	Entity Fee ($)	Small Fee Code	Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	380	216	190	Extension for reply within second month	
117	870	217	435	Extension for reply within third month	
118	1,360	218	680	Extension for reply within fourth month	
128	1,850	228	925	Extension for reply within fifth month	
119	300	219	150	Notice of Appeal	
120	300	220	150	Filing a brief in support of an appeal	
121	260	221	130	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,210	241	605	Petition to revive - unintentional	
142	1,210	242	605	Utility issue fee (or reissue)	
143	430	243	215	Design issue fee	
144	580	244	290	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	240	126	240	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	690	246	345	Filing a submission after final rejection (37 CFR 1.129(a))	
149	690	249	345	For each additional invention to be examined (37 CFR 1.129(b))	

Other fee (specify) :
Other fee (specify) :
*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($)

SUBMITTED BY				Complete (if applicable)	
Typed or Printed Name	Robert W. Esmond			Reg. Number	32,893
Signature	Robert W. Esmond	Date	September 20, 2000	Deposit Acct. User ID	

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Nucleic Acid Marker Ladder For Estimating Mass

Field of the Invention

The present invention is in the field of molecular biology and specifically relates to the technique of gel electrophoresis of nucleic acid fragments.

Background of the Invention

Gel electrophoresis of nucleic acid is a well known technique in molecular biology. Nucleic acid molecules are separated on the basis of size (length or molecular weight), and conformation (linear *vs.* nicked circles *vs.* covalently closed circles). For a given conformation, electrophoretic mobility is inversely related to size.

Conventional agarose gel electrophoresis is commonly used for the separation of nucleic acid fragments within a practical resolution limit of 50 kbp (Cantor, C.R. and Schimmel, P.R. (1980) *Biophysical Chemistry*, Vol. III, pp. 1012-1036, Freeman, San Francisco; and Maniatis, T. *et al.* (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). A method called pulsed field gel electrophoresis (PFGE) has been developed to provide separation of DNA molecules up to 2 Mbp (Schwartz, D.C. *et al.* (1983) *Cold Spring Harbor Symp. Quant. Biol. 47*:189-195.; and Schwartz, D.C. and Cantor, C.R. (1984) *Cell 37*:67-75).

A number of mixtures of nucleic acid fragments ("ladders") are commercially available that can be used as markers for determining or estimating the sizes of nucleic acid molecules during gel electrophoresis. One type of ladder is constructed by digesting plasmids or bacteriophage with one or more restriction enzymes. The size of the marker fragments

will depend upon the natural location of the restriction enzyme site within the molecule to be digested and will produce a quasi-random size distribution. For example digestion of bacteriophage λ (*lambda*) with *Hind*III produces fragments of 23,130, 9,416, 6557, 4361, 2322, 2027, 564, and 125 base pairs (bp) (See Cat. No. 5612SA, Life Technologies, Inc. 1992 catalogue, Gaithersburg, MD, p. 318).

Alternatively, a ladder may comprise fragments which vary linearly with molecular weight, i.e. adjacent bands may differ by about 1000 base pairs (e.g "1 Kb DNA Ladder", See Cat. No. 5615SA, Life Technologies, Inc. 1992 catalogue, Gaithersburg, MD, p. 323), 100 base pairs (e.g "100 bp DNA Ladder", See Cat. No. 5628SA, Life Technologies, Inc. 1992 catalogue, Gaithersburg, MD, p. 322), or 123 bp (e.g "123 bp DNA Ladder", See Cat. No. 5613SA, Life Technologies, Inc. 1992 catalogue, Gaithersburg, MD, p. 323). Some ladders have been constructed and sold that are logarithmically spaced ("GenePrint™", cat. no. DG1911, Promega, Madison, WI).

Nucleic acid is visualized in agarose gels following electrophoresis by staining with the florescent dye ethidium bromide (Sharp *et al.* (1973) *Biochemistry 12:*3055). Ethidium bromide contains a planar group that intercalates between nucleic acid bases. The fixed position of this planar group and its close proximity to the nucleic acid bases cause the ethidium bromide bound to the nucleic acid to display an increased fluorescent yield compared to that of ethidium bromide in free solution (See Sambrook *et al.* (1989) Molecular Cloning, 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, page 6.15). The molecular mass of a nucleic acid fragment can be determined following agarose gel electrophoresis and ethidium bromide staining by comparing the intensity of the florescence of a fragment of unknown molecular mass with the intensity of a similarly sized fragment of known molecular mass.

Summary of the Invention

In general, the present invention provides a nucleic acid marker ladder. More specifically, the present invention provides a nucleic acid marker ladder consisting essentially of a restriction endonuclease digest wherein

(a) the nucleic acid restriction endonuclease digest is a collection of nucleic acid fragments resulting from the complete digestion of one or more nucleic acids by one or more restriction endonucleases;

(b) the restriction endonuclease digest contains at least 3 fragments; and

(c) the size of the fragments in base pairs is a multiple of an integer, wherein the integer is 10 or more.

The present invention also provides a nucleic acid marker kit comprising a carrier means having in close confinement therein at least one container means where a first container means contains the above-described nucleic acid marker ladder.

The present invention also provides a method of preparing a nucleic acid marker ladder comprising:

(a) generating at least two polymerase chain reaction (PCR) products wherein each product is generated from a template comprising a restriction endonuclease site and a primer comprising the restriction endonuclease site in the template;

(b) joining the PCR products to produce one or more nucleic acid molecules; and

(c) completely digesting the nucleic acid molecules with at least one restriction endonuclease

wherein a nucleic acid marker ladder is produced wherein the ladder contains at least 3 fragments and the size of the fragments in base pairs is a multiple of an integer, wherein the integer is 10 or more.

Further objects and advantages of the present invention will be clear from the description that follows.

Brief Description of the Figures

FIGURE 1. A plasmid map of pML1 (Mass Ladder).

FIGURE 2. Restriction enzyme digest of pML1. Lane 1. 100 bp ladder (See Cat. No. 5628SA, Life Technologies, Inc. 1992 catalogue, Gaithersburg, MD, p. 322); Lanes 2, 4-6, 8-9. *SspI* digest of pML1.

Detailed Description of the Invention

The present invention relates to a nucleic acid marker ladder.

In one embodiment, the present invention relates to a nucleic acid marker ladder consisting essentially of a restriction endonuclease digest, wherein

(a) the nucleic acid restriction endonuclease digest is a collection of nucleic acid fragments resulting from the complete digestion of one or more nucleic acids by one or more restriction endonucleases;

(b) the restriction endonuclease digest contains at least 3 fragments; and

(c) the size of the fragments in base pairs is a multiple of an integer, wherein the integer is 10 or more. In one preferred embodiment the integer is 10. In another preferred embodiment, the integer is 25. In yet another preferred embodiment, the integer is 50. In a further embodiment, the integer is 100. In another preferred embodiment, the collection of nucleic acid fragments results from digestion of a nucleic acid by one restriction endonuclease. In a further preferred embodiment, the nucleic acid is DNA. One skilled in the art would recognize that the size

of the fragments can be approximately a multiple of an integer. For example, the fragment's size can be 101, 201, 301, and 401 bp.

In another embodiment, the present invention relates to a nucleic acid marker kit comprising a carrier means having in close confinement therein at least one container means where a first container means contains the above-described nucleic acid marker ladder.

A restriction endonuclease (also restriction enzyme) is an enzyme that has the capacity to recognize a specific base sequence (usually 4, 5, or 6 base pairs in length) in a DNA molecule, and to cleave the DNA molecule where this sequence appears. For example, *Eco*RI recognizes the base sequence GAATTC/CTTAAG.

The DNA molecules produced by digestion with a restriction endonuclease are referred to as restriction fragments. Any given genome, plasmid or phage may be digested by a particular restriction endonuclease into a discrete set of restriction fragments.

The most commonly used analytical method (though not the only one) for fractionating double-stranded DNA molecules on the basis of size is agarose gel electrophoresis. The principle of this method is that DNA molecules migrate through the gel as though it were a sieve that retards the movement of the largest molecules to the greatest extent and the movement of the smallest molecules to the least extent. Note that the smaller the DNA fragment, the greater the mobility under electrophoresis in the agarose gel. The DNA fragments fractionated by agarose gel electrophoresis can be visualized directly by a staining procedure if the number of fragments included in the pattern is small. Preferably, the nucleic acid-containing agarose gel is stained with ethidium bromide.

As will be understood by those of skill in the art, the nucleic acid molecules used to form the marker ladder are preferably any linear or circular DNA which is cleavable by a restriction enzyme. For example, the nucleic acid may be chromosomes, plasmids, cosmids or viral nucleic

acid. Preferably, the nucleic acid molecules are plasmid or viral molecules and derivatives thereof. The nucleic acid present in the plasmid or viral molecule may include exogenous nucleic acid which has been joined to produce the plasmid or viral molecule. In one preferred embodiment, the nucleic acid is DNA.

In another embodiment, the present invention relates to a method of preparing a nucleic acid marker ladder comprising:

(a) generating at least two polymerase chain reaction (PCR) products wherein each product is generated from a template comprising a restriction endonuclease site and a primer comprising the restriction endonuclease site in the template;

(b) joining the PCR products to produce one or more nucleic acid molecules; and

(c) completely digesting the nucleic acid molecules with at least one restriction endonuclease

wherein a nucleic acid marker ladder is produced wherein the ladder contains at least 3 fragments and the size of the fragments in base pairs is a multiple of an integer, wherein the integer is 10 or more.

To construct a nucleic acid molecule which when digested with a restriction endonuclease produces the marker ladder of the present invention, the number of fragments in the restriction endonuclease digest and the desired size of the fragments are selected. In one preferred embodiment, the restriction endonuclease digest contains at least three fragments. In another preferred embodiment, the restriction endonuclease digest contains 3, 4, 6, 8 or 10 fragments. In a further preferred embodiment, the restriction endonuclease digest contains 6 fragments.

The size of the fragments in base pairs is preferably selected to be a multiple of an integer, wherein the integer is 10 or more. In one preferred embodiment, the integer is 10, 25, 50, or 100. In another preferred embodiment, the integer is 100.

Preferably, one of the fragments contains an origin of replication (for example, *ori*) such that the nucleic acid molecule may autonomously replicate within a cell. It is also preferable that one of the fragments contains a selectable or screenable marker. The origin of replication and the marker may be present on the same fragment. Transformants containing this DNA fragment may be cultured and selected with a selection agent corresponding to the selectable marker.

The nucleic acid molecule is preferably constructed from polymerase chain reaction (PCR) products. The polymerase chain reaction provides a method for selectively increasing the concentration of a nucleic acid molecule having a particular sequence even when that molecule has not been previously purified and is present only as a single copy in a particular sample. The method can be used to amplify single or double stranded nucleic acid. Reviews of the polymerase chain reaction are provided by Mullis, K.B., *Cold Spring Harbor Symp. Quant. Biol. 51:*263-273 (1986); Saiki, R.K., *et al., Bio/Technology 3:*1007-1012 (1985); Mullis, K.B., *et al., Methods in Enzymology 155:*350 (1987); Mullis, K., *et al.*, U.S. Patent No. 4,683,202; Erhlich, H., U.S. Patent No. 4,582,788; and Saiki, R., *et al.*, U.S. Patent No. 4,683,194.

In one preferred embodiment, a PCR product has the desired restriction endonuclease site internally. In another preferred embodiment, a PCR product has the desired restriction endonuclease site at the right and/or left end of the PCR product. In a further preferred embodiment, the PCR product has the desired restriction endonuclease site internally and at the right or left end of the PCR product.

The PCR product is preferably generated from a naturally occurring template. The template is preferably plasmid, phage, or plant, animal, or bacterial genomic DNA. The template preferably has an internal restriction endonuclease site. The desired restriction endonuclease site at the right or

left end of the PCR product is preferably generated by a restriction endonuclease site in the PCR primer.

The spacing of the PCR priming sites and the naturally occurring restriction sites is preferably arranged so that when the PCR products are joined together, the nucleic acid molecule so formed when harvested from *E. coli* can be cut with the desired restriction endonuclease to produce the digest of the present invention.

For example, if a ladder of 6 fragments is preferred, three PCR products may be joined together. Product 1 would preferably contain joinable ends A and C, wherein C contains the desired restriction endonuclease site. Product 1 would also preferably contain the desired restriction endonuclease site (B) internally.

Product 1 A B C

Product 2 would preferably contain joinable ends D and F, wherein F contains the desired restriction endonuclease site. Product 2 would also preferably contain the desired restriction endonuclease site (E) internally.

Product 2 D E F

Product 3 would preferably contain joinable ends G and I, wherein I contains the desired restriction endonuclease site. Product 3 would also preferably contain the desired restriction endonuclease site (H) internally.

Product 3 G H I.

The primer ends are constructed such that C is joinable to D, F is joinable to G, and I is joinable to A. The primer ends A, C, D, F, G, and I are variable and depend upon the desired size of the fragments present in the digest.

The PCR products can be joined together using ligase (preferably, *E. coli* DNA ligase, Cat. No. 8052SA, Life Technologies, Inc., Gaithersburg, MD) to produce a nucleic acid molecule. The molecule is then digested with at least one restriction endonuclease wherein a nucleic acid marker ladder is produced wherein the ladder contains at least 3

fragments and the size of the fragments in base pairs is a multiple-of-an integer, wherein the integer is 10 or more. Preferably, one restriction endonuclease is used. The six fragments produced from digestion of the above-described molecule are B-C, C-E, E-F, F-H, H-I, and I-B.

In the examples that follow, DNA from three sources was joined together to form a nucleic acid which when cleaved with a restriction enzyme produces a marker ladder of 6 fragments wherein the fragments are multiples of 100.

The present invention is useful as a standard to be used during electrophoresis. A marker ladder wherein the size of the fragments is a multiple of an integer (preferably an integer of 10 or more) is extremely convenient and easy to use since one skilled in the art can quickly calculate the size of an unknown nucleic acid fragment.

However, the marker ladder of the present invention not only allows one to size a nucleic acid but also to determine the mass of the nucleic acid. The molecular mass of a nucleic acid fragment can be determined following agarose gel electrophoresis and ethidium bromide staining by comparing the intensity of the florescence of a fragment of unknown molecular mass with the intensity of a similarly sized fragment of known molecular mass. The molecular mass is easily determined because all of the fragments derive from a single nucleic acid molecule.

The present invention is described in further detail in the following non-limiting examples.

Example 1

Construction of an SspI Marker Ladder

A marker ladder was constructed from three PCR products. Each PCR product has an *Ssp* I at its right end, and an internal *Ssp* I site (naturally occurring in the DNA template). The spacing of the priming

sites and the naturally occurring restriction sites was arranged so that when the three PCR fragments were annealed together (uracil bases in the 5′ ends of the primers, treated with uracil DNA glycosylase (Life Technologies, Inc., Gaithersburg, MD); 3′ ends anneal; U.S. Patent Nos. 5,137,814 and 5,229,283) to form a circular molecule, the plasmid so formed when harvested from *E. coli* can be cut (to completion) into six *Ssp* I fragments, all of which are multiples of 100 bp.

Three polymerase chain reactions were performed with each of the following three sets of primers:

A Spacer pUC coord. 525

pUC left: 5′ [auc uga ccu cau] [aat tta] [cgg aag cat aaa gtg taa agc ct] 3′

B′ *Ssp* I pUC coord. 2600

pUC right: 5′ [agu cac agc uau] [aat att] [gga aat gtg cgc gga acc cc] 3′

B Spacer Ad2 coord. 32645

Adeno2 left: 5′[aua gcu gug acu] [aat tta] [cta gtg aat cca cag aaa cta gc] 3′

C′ *Ssp* I Ad2 coord. 34620

Adeno2 right: 5′ [aca ucu gga cuu] [aat att] [aga cat att gat aag gtg gcg ag] 3′

C Spacer SV40 coord. 1127

SV40 Left: 5′ [aag ucc aga ugu] [aat tta] [ggg aca gtt tgg caa ggt ttt ta] 3′

A′ *Ssp* I SV40 coord. 1702

SV40 right: 5′ [aug agg uca gau] [aat att] [taa gcc ttt ttg atg ttc atc agg] 3′

Each 50 μl polymerase chain reaction contained 0.3 μM of each primer (reaction (a) contained the pUC primers; reaction (b) contained the Adeno2 primers; and reaction (c) contained the SV40 primers), 1 μl AmpliTaq (Perkin Elmer Cetus), 1 ng of template DNA (SV40 cut with *Kpn* I; Adenovirus 2; or pUC19 cut with *Eco*RI (all DNAs from Life

Technologies, Inc., Gaithersburg, MD)) and 1 X PCR buffer (50 mM KCl, 10 mM Tris HCl pH 8.3, 1.5 mM $MgCl_2$, 5 mM 2-mercaptoethanol). Cycling conditions were 94° 5 min; thirty cycles of 94° 30 sec, 55° 30 sec. 72° 2 min; and hold at 0°.

UDG cloning was used to clone the PCR products. One μl of each PCR product was combined with 14 μl water, 2 μl 10 X PCR buffer, and 1 μl = 1 unit UDG (Life Technologies, Inc., Gaithersburg, MD), incubated 37° 30 min, and transformed 1 μl into DH5 alpha cells (Life Technologies, Inc., Gaithersburg, MD). About 2500 colonies were obtained. Twelve colonies were picked for minipreps. Of these twelve, eleven gave the expected 6 *Ssp* I fragments of the expected size.

One of these plasmids was chosen and named "pML1". This plasmid is exactly 4700 bp, and contains six *Ssp* I (blunt) fragments, of sizes 2000, 1200, 800, 400, 200 and 100 bp. Thus, when 470 ng of this complete digest are applied to a gel, the six fragments contain 200, 120, 80, 40, 20 and 10 ng of DNA.

Example 2

Construction of a NotI Marker Ladder

A marker ladder can be constructed from PCR products. The sizes of the resulting DNAs can be selected by positioning the PCR primers appropriately. In addition, the incorporation of restriction sites in the primers, which are absent in the template molecules that are amplified during PCR, allows the ends of the PCR products to be cleaved with cognate restriction enzymes. If multiple PCR product DNAs are joined together and transformed into bacteria, and if one or more of the PCR products contains an origin of replication and a selection marker, the joined molecule can be recovered from the bacteria as a recombinant molecule.

Digestion of the molecule with the restriction enzyme whose sites have been placed in the primers yields the desired fragments. By using a variety of restriction enzyme sites to link PCR products, and a different restriction enzyme to cleave the resulting recombinant plasmid, multiple DNAs can be joined together in a particular order to give the desired product.

Using these principles, a marker plasmid can be constructed as follows. Three PCR products are synthesized. Terminal restriction sites allow joining the fragments in a unique order and orientation. *NotI* sites will be used to release the three desired fragments from the resulting plasmid. Spacers at the ends of the PCR products ensure efficient restriction enzyme cutting.

PCR product I, 2026 bp from pUC:	
Left primer:	Nsp V Not I pUC19 coord. 637 - 656 5 10 nt spacer/tt^cgaa/gc^ggccgg/taa tga atc ggc caa cgc gc 3
Right primer:	BssH I pUC 19 coord. 2622 - 2603 5 10 nt spacer/g^cgcgc/ga cgt cag gtg gca ctt ttc 3

PCR product II, 1026 bp from SV40 DNA:	
Left primer:	Mlu I Not I SV40 coord. 108 - 129 5 10 nt spacer/a^cgcgt/gc^ggccgc/ggt tgc tga cta att gag atg c 3
Right primer:	BamH I SV40 coord. 1093 - 1074 5 10 nt spacer/g^gatcc/gtg agg tga gcc tag gaa tg 3

PCR product III, 526 bp from adenovirus 2:	
Left primer:	Bgl II Not I Ad2 coord. 1022 - 1041 5' 10 nt spacer/a^gatct/gc^ggccgc/ggt ctt gtc att atc acc gg 3'
Right primer:	Cla I Ad2 coord. 1507-1488 5' 10 nt spacer/at^cgat/gtt gcc cag act cgt taa gc 3'

Each PCR product is digested with the two enzymes that cut at each end, i.e., I + Nsp V and BssH II, II with Mlu I and BamH I, and III with Bgl II and Cla I. The three digested products are mixed and joined, and the products are digested with the six restriction enzymes prior to

transformation into *E. coli*. The amplified fragments must be chosen to lack internal sites for these enzymes. The only clones that should be produced by this process should be 3500 bp plasmids which can be cleaved with Not I to give three fragments of 2000, 1000, and 500 bp. Electrophoresis of 350 ng of this digest will give three bands containing 200, 100, and 50 ng DNA, respectively.

* * * * *

All publications mentioned hereinabove are hereby incorporated in their entirety by reference.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention and appended claims.

What Is Claimed Is:

1. A nucleic acid marker ladder consisting essentially of a restriction endonuclease digest, wherein

(a) the nucleic acid restriction endonuclease digest is a collection of nucleic acid fragments resulting from the complete digestion of one or more nucleic acids by one or more restriction endonucleases;

(b) the restriction endonuclease digest contains at least 3 fragments; and

(c) the size of the fragments in base pairs is a multiple of an integer, wherein the integer is 10 or more.

2. The nucleic acid marker ladder according to claim 1, wherein the integer is 10.

3. The nucleic acid marker ladder according to claim 1, wherein the integer is 25.

4. The nucleic acid marker ladder according to claim 1, wherein the integer is 50.

5. The nucleic acid marker ladder according to claim 1, wherein the integer is 100.

6. The nucleic acid marker ladder according to claim 1, wherein the collection of nucleic acid fragments results from digestion of a nucleic acid by one restriction endonuclease.

7. A nucleic acid marker kit comprising a carrier means having in close confinement therein at least one container means where the first container means contains a nucleic acid marker ladder consisting essentially of a restriction endonuclease digest, wherein

(a) the nucleic acid restriction endonuclease digest is a collection of nucleic acid fragments resulting from the complete digestion of one or more nucleic acids by one or more restriction endonucleases;

(b) the restriction endonuclease digest contains at least 3 fragments; and

(c) the size of the fragments in base pairs is a multiple of an integer, wherein the integer is 10 or more.

8. The nucleic acid marker kit according to claim 7, wherein the integer is 10.

9. The nucleic acid marker kit according to claim 7, wherein the integer is 25.

10. The nucleic acid marker kit according to claim 7, wherein the integer is 50.

11. The nucleic acid marker kit according to claim 7, wherein the integer is 100.

12. The nucleic acid marker kit according to claim 7, wherein the collection of nucleic acid fragments results from digestion of a nucleic acid by one restriction endonuclease.

13. A method of preparing a nucleic acid marker ladder comprising:

(a) generating at least two polymerase chain reaction (PCR) products wherein each product is generated from a template comprising a restriction endonuclease site and a primer comprising the restriction endonuclease site in the template;

(b) joining the PCR products to produce a nucleic acid molecule; and

(c) completely digesting one or more nucleic acid molecules with at least one restriction endonuclease

wherein a nucleic acid marker ladder is produced wherein the ladder contains at least 3 fragments and the size of the fragments in base pairs is a multiple of an integer, wherein the integer is 10 or more.

14. A method of using a nucleic acid marker ladder to estimate the mass of a nucleic acid comprising:

(a) electrophoresing a known amount of the marker ladder of claim 1 and an unknown amount of said nucleic acid on an agarose gel; and

(b) comparing the mass of said marker ladder with the mass of said nucleic acid.

Abstract of the Disclosure

The invention relates to a nucleic acid marker ladder which is a restriction endonuclease digest, wherein a nucleic acid restriction endonuclease digest is a collection of nucleic acid fragments resulting from complete digestion of one or more nucleic acids by one or more restriction endonucleases; the restriction endonuclease digest contains at least 3 fragments; and the size of the fragments in base pairs is a multiple of an integer, wherein the integer is 10 or more.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
James L. HARTLEY	Art Unit: To be assigned
Appl. No. To be assigned (Continuation of U.S. Appl. No. 09/114,911; Filed: July 14, 1998)	Examiner: To be assigned
Filed: HEREWITH	Atty. Docket: 0942.2570003/RWE/DRM
For: **Nucleic Acid Markers Ladder for Estimating Mass**	

Letter to PTO Draftsman: Submission of Formal Drawings

Commissioner for Patents
Washington, D.C. 20231

Sir:

Submitted herewith are 2 sheets of formal drawings with Figures 1 and 2, corresponding to the informal drawings submitted with the above-captioned application. The application number, group art unit and attorney docket number appear on the back of each sheet. Acknowledgment of the receipt, approval, and entry of these formal drawing(s) into this application is respectfully requested.

It is not believed that an extension of time is required, other than any already provided herewith. However, if an extension of time is needed to prevent abandonment of the application, then such extension of time is hereby petitioned. The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036. A duplicate copy of this Letter is enclosed.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Robert W. Esmond

Robert W. Esmond
Attorney for Applicant
Registration No. 32,893

Date: September 20, 2000

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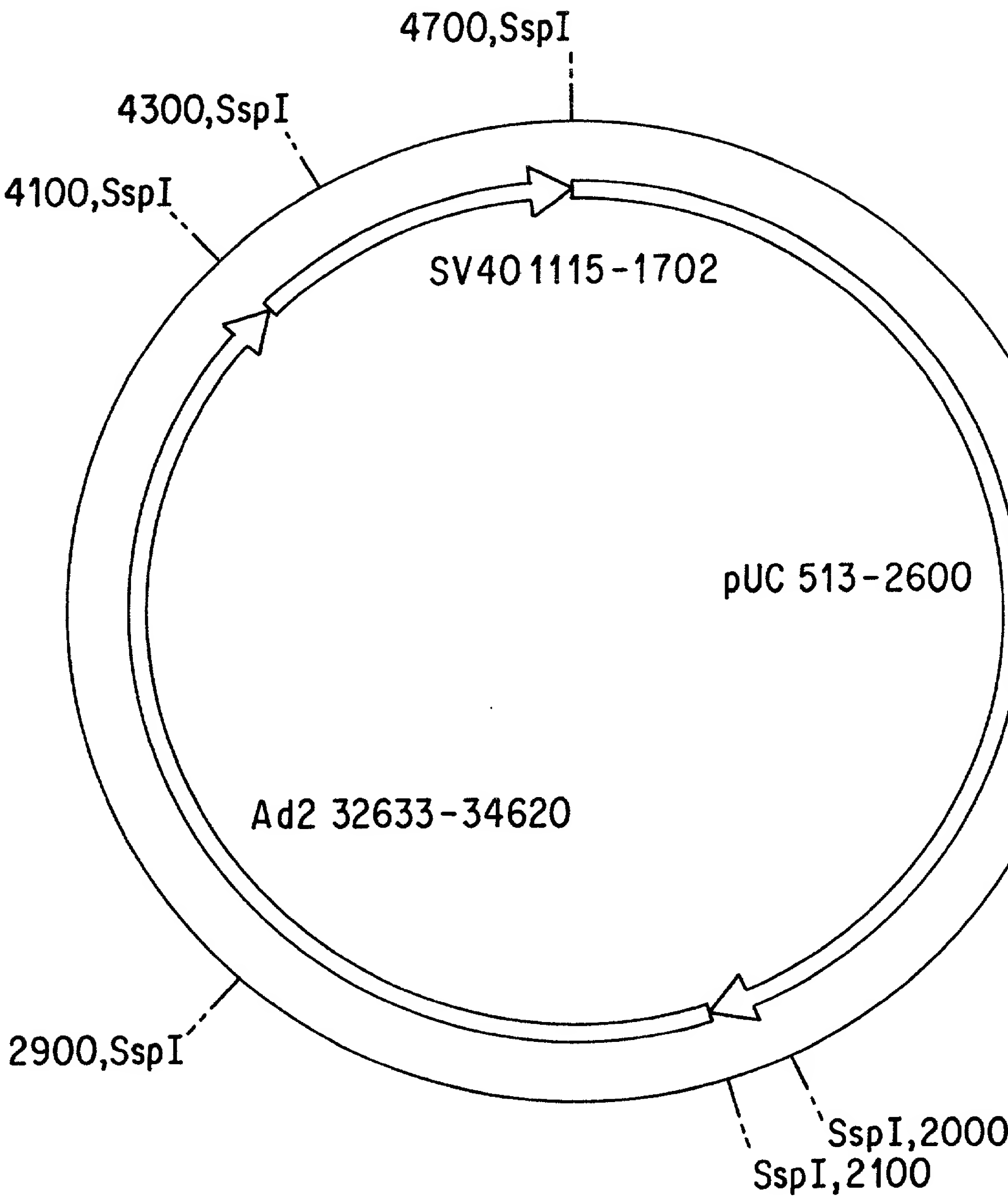
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SspI,2100

FIG.1

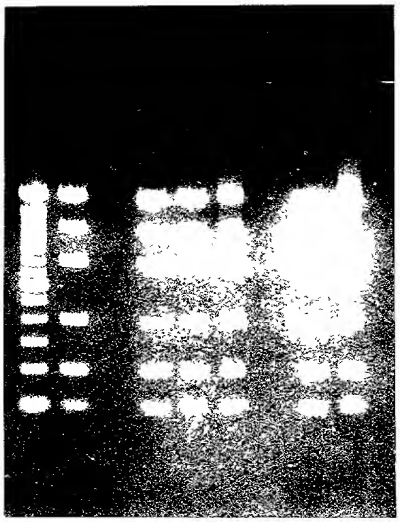

FIG.2

DUPLICATE

Declaration and Power of Attorney for Patent Application
English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

NUCLEIC ACID MARKER LADDER FOR ESTIMATING MASS

the specification of which

(check one)

☒ is attached hereto.

☐ was filed on _____
Serial No. _____
amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed
(Number)	(Country)	(Day Month Year Filed)	☐ Yes ☐ No
(Number)	(Country)	(Day Month Year Filed)	☐ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Serial No.)	(Filing Date)	(Status)
(Application Serial No.)	(Filing Date)	(Status) (patented; pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (*list name and registration number*)

Robert G. STERNE, Esq., Reg. No. 28,912; Edward J. KESSLER, Esq., Reg. No. 25,688; Jorge A. GOLDSTEIN, Esq., Reg. No. 29,021; Samuel L. FOX, Esq., Reg. No. 30,353; David K. S. CORNWELL, Esq., Reg. No. 31,944; Robert W. ESMOND, Reg. No. 32,893; Tracy-Gene G. DURKIN, Esq., Reg. No. 32,831 and Michele A. CIMBALA, Esq., Reg. No. 33,851

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Inventor's signature	James L. Hartley — 10/27/93 Date
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Citizenship	United States
Post Office Address	same as residence
Full name of second joint inventor, if any	
Second Inventor's signature	Date
Residence	
Citizenship	
Post Office Address	

(Supply similar information and signature for subsequent joint inventors, if any).

0942257.DEC

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Hartley, James L.

(ii) TITLE OF INVENTION: Nucleic Acid Marker Ladder For Estimating Mass

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Sterne, Kessler, Goldstein & Fox
(B) STREET: 1100 New York Avenue, Suite 600
(C) CITY: Washington
(D) STATE: D.C.
(E) COUNTRY: USA
(F) ZIP: 20005

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 09/114,911
(B) FILING DATE: 14-JUL-1998
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/893,523
(B) FILING DATE: 11-JUL-1997

(viii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/142,124
(B) FILING DATE: 28-OCT-1993

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: McPhail, Donald R.
(B) REGISTRATION NUMBER: 35,811
(C) REFERENCE/DOCKET NUMBER: 0942.2570002

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (202) 371-2600
(B) TELEFAX: (202) 371-2540

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AUCUGACCUC AUAATTTACG GAAGCATAAA GTGTAAAGCC T 41

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AGUCACAGCU AUAATATTGG AAATGTGCGC GGAACCCC 38

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AUAGCUGUGA CUAATTTACT AGTGAATCCA CAGAAACTAG C 41

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ACAUCUGGAC UUAATATTAG ACATATTGAT AAGGTGGCGA G 41

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAGUCCAGAU GUAATTTAGG GACAGTTTGG CAAGGTTTTT A 41

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AUGAGGUCAG AUAATATTTA AGCCTTTTTG ATGTTCATCA GG 42

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TTCGAAGCGG CCGGTAATGA ATCGGCCAAC GCGC 34

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCGCGCGACG TCAGGTGGCA CTTTTC 26

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACGCGTGCGG CCGCGGTTGC TGACTAATTG AGATGC 36

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGATCCGTGA GGTGAGCCTA GGAATG 26

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGATCTGCGG CCGCGGTCTT GTCATTATCA CCGG 34

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATCGATGTTG CCCAGACTCG TTAAGC 26